Journal of Medical Virology 58:59–62 (1999)

Influence of Maternal CD4 Levels on the Predictive Value of Virus Load Over Mother-to-Child Transmission of Human Immunodeficiency Virus Type 1 (HIV-1)

Cinzia Mazza,[1] Antonella Ravaggi,[1] Anna Rodella,[1] Marzia Duse,[2] Debora Padula,[3] Manuela Lomini,[4] Francesco Castelli,[5] Susanna Bresciani,[1] Alberto Albertini,[6] Elisabetta Cariani,[1]* and the Study Group for Vertical Transmission

[1]III Laboratory of Clinical Chemistry, Hospital of Brescia, Brescia, Italy
[2]Institute of Paediatrics, School of Medicine, University of Brescia, Brescia, Italy
[3]Department of Neonatal Paediatrics, Hospital of Brescia, Brescia, Italy
[4]II Department of Obstetrics and Gynaecology, Hospital of Brescia, Brescia, Italy
[5]Institute of Infectious Diseases, School of Medicine, University of Brescia, Brescia, Italy
[6]Institute of Chemistry, School of Medicine, University of Brescia, Brescia, Italy

Forty-four anti-HIV seropositive pregnant women were enrolled in a study of maternal factors related to mother-to-infant human immunodeficiency virus type 1 (HIV-1) transmission. HIV-1 infection was documented in 11 of 45 infants (24.4%). Obstetric factors, maternal CD4 counts, and disease stage were not related to the risk of transmission. HIV-1 RNA levels at delivery were significantly higher in mothers who transmitted the infection ($P = .024$). A strong relationship between viral load and risk of transmission was observed in women with stage A1 ($P = .006$), but not in those with stages A2–A3. These results suggest that vertical transmission of HIV-1 is multifactorial and that viral load plays a major role in mothers with early-stage HIV-1 infection. ***J. Med. Virol. 58:59–62, 1999.***

KEY WORDS: HIV RNA; HIV DNA; quantitative polymerase chain reaction (PCR)

Mother-to-infant transmission of human immunodeficiency virus type 1 (HIV-1) is a major health problem worldwide. Previous studies have suggested that there are multiple determinants of HIV-1 transmission, including maternal immune response, obstetric factors, and characteristics of the infecting virus [Weiser et al., 1994; European Collaborative Study, 1996; Landesman et al., 1996; Rodriguez et al. 1996]. Recently, the HIV-1 RNA load has been indicated as predictive of the risk of vertical-perinatal transmission [Fang et al., 1995; Dickover et al., 1996], but the strength of the association between RNA level and transmission varies among different studies [Dickover et al. 1996; Sperling et al., 1996; Burns et al., 1997; Cao et al., 1997].

The present study examined the predictive value of maternal HIV-1 RNA levels determined by quantitative reverse transcription-polymerase chain reaction (RT-PCR) on the risk of transmission from mothers with different disease stage.

PATIENTS AND METHODS

Study Population

Forty-four women with anti-HIV (40 Caucasian and 4 African, mean age 27.6 ± 4.2 years) were enrolled prospectively between November 1992 and August 1996, either during pregnancy or at delivery, for the study of mother-to-infant HIV-1 transmission. All women were informed of the aim of the study. Three mothers were treated with zidovudine.

Infection in the child was defined by p24 antigenaemia and/or presence of HIV DNA on two or more occasions, accompanied by persistence of antibodies beyond 18 months of age in infants with longer follow-up [European Collaborative Study, 1992]. The study protocol included determination of maternal p24 antigen, HIV-1 DNA and HIV-1 RNA (quantitative assessment) at the time of delivery.

Study Group for Vertical Transmission: A. Gussago,[1] A. Mabellini,[1] R. Marinelli,[4] S. Pecorelli,[4] G. Pizzocolo,[1] E. Prati,[4] M. Puoti,[5] V. Putzolu,[5] F. Quaglia,[4] M. Spandrio,[3] and S. Timpano[2].

*Correspondence to: Elisabetta Cariani, III Laboratorio Analisi, A.O. Spedali Civili di Brescia, P. le Spedali Civili, 1, 25123 Brescia, Italy.

Accepted 17 September 1998

TABLE I. Characteristics of Mothers

	VT	NVT	Total	*P*
n	11	33	44	
Age (years) (mean ± SD)	29.8 ± 4.9	26.8 ± 3.8	27.6 ± 4.2	NS
Risk factors [*n* (%)]				
IVDU	7 (63.6)	22 (66.7)	29 (66)	NS
T	0	1 (3)	1 (2)	NS
S	4 (36.4)	7 (21.2)	11 (25)	NS
NN	0	3 (9.1)	3 (7)	NS
Delivery [*n* (%)]				
V	9 (81.8)	18 (55)	27 (61.4)	NS
CS	2 (18.2)	15 (45)	17 (38.6)	NS
CD4+/mm^3 (mean ± SD)	397 ± 245	539 ± 280	504 ± 276	NS
CDC class [*n* (%)]				
A1	5 (45.4)	17 (51.4)	22 (50)	NS
A2	3 (27.3)	14 (42.4)	17 (38.6)	NS
A3	3 (27.3)	2 (6.1)	5 (11.4)	NS
p24 Ag				
n positive (%)	3 (27.3)	3 (9.1)	6 (13.6)	NS
HIV DNA				
n positive (%)	8 (72.7)	23 (69.7)	31 (70.5)	NS
HIV RNA				
n tested	10	33	43	NS
n positive (%)	10 (100)	30 (90.9)	40 (93)	NS
Median[a]	4.27	3.59	3.85	.024
Range[a]	3–6.33	2.3–5.1	2.3–6.33	

VT/NVT, mothers involved/not involved in vertical transmission; SD, standard deviation; NS, not significant; IVDU, intravenous drug use; T, transfusion; S, sexual activity with multiple partners or with seropositive partner; NN, unknown; V, vaginal delivery, CS cesarean section.
[a]log copies/ml.

Serological Tests

Anti-HIV antibodies were determined in duplicate by the HIV-1/HIV-2 enzyme-linked immunosorbent assay (ELISA) test System (Ortho Diagnostic Systems, Raritan, NJ) and the recombinant HIV-1/HIV-2 enzyme immunoassay (EIA) (Abbott Diagnostics, Abbott Park, IL) and confirmed by the HIV blot 2.2 (Diagnostic Biotechnology, Singapore, Malaysia).

The detection of HIV-1 p24 antigen was carried out by the HIV-1 Antigen EIA (Abbott Diagnostics).

Amplification of HIV DNA and RNA

All samples were stored at −80°C until used. HIV-1 DNA was determined in duplicate by PCR using the Amplicor HIV kit (Roche Diagnostic Systems, Branchburg, NJ). The determination of the HIV-1 RNA copy number was carried out on plasma samples by the Amplicor HIV Monitor assay (Roche Diagnostic Systems) following the supplier's instructions. Briefly, RNA was extracted from 100 μl of plasma with lysis buffer containing the internal standard, and diluted in 1 ml of specimen diluent. RT-PCR was then performed on 50 μl of diluted RNA in a final volume of 100 μl. Serial dilutions of the amplified products were detected on microtiter plates coated with probes specific for template and internal standard.

Statistical Analysis

The characteristics of patients were compared by parametric or nonparametric tests as appropriate, using the EPI.INFO 5.0 statistical package (Centers for Disease Control, Atlanta, GA). The relationship between CD4 cell counts and HIV-1 RNA levels was analyzed by the Spearman rank order correlation coefficient. A P value of .05 or less was considered to be statistically significant.

RESULTS

The 45 infants (including one set of twins) born to the 44 HIV-1-infected mothers included in this study were monitored for 6–48 months (mean 18.3 ± 8.9 months). Diagnosis of HIV infection was made in 11 infants (24.4%): 7 girls and 4 boys.

To analyze the possible risk factors for vertical transmission, the mothers who transmitted HIV-1 were compared with those who did not (Table I). Risk factors for infection and type of delivery were not related statistically to the occurrence of vertical transmission. None of the mothers breast-fed. CD4 cell counts and stage of HIV-1 infection, determined by the Revised Centers for Disease Control system [1992], did not differ between the two groups of mothers, although a trend was observed to lower CD4 levels in mothers that transmitted HIV-1.

At delivery, 6 women (13.6%) were positive for p24 antigen and 31 (70.5%) for HIV-1 DNA. Plasma samples adequately stored for HIV-1 RNA testing were available for 43 women, 40 of whom (93%) had positive results. The prevalence of p24 antigen and of HIV-1 DNA did not differ between transmitting and nontransmitting mothers, whereas HIV-1 RNA levels were significantly higher in mothers who transmitted the infection to their babies ($P = .024$) (Table I). The dis-

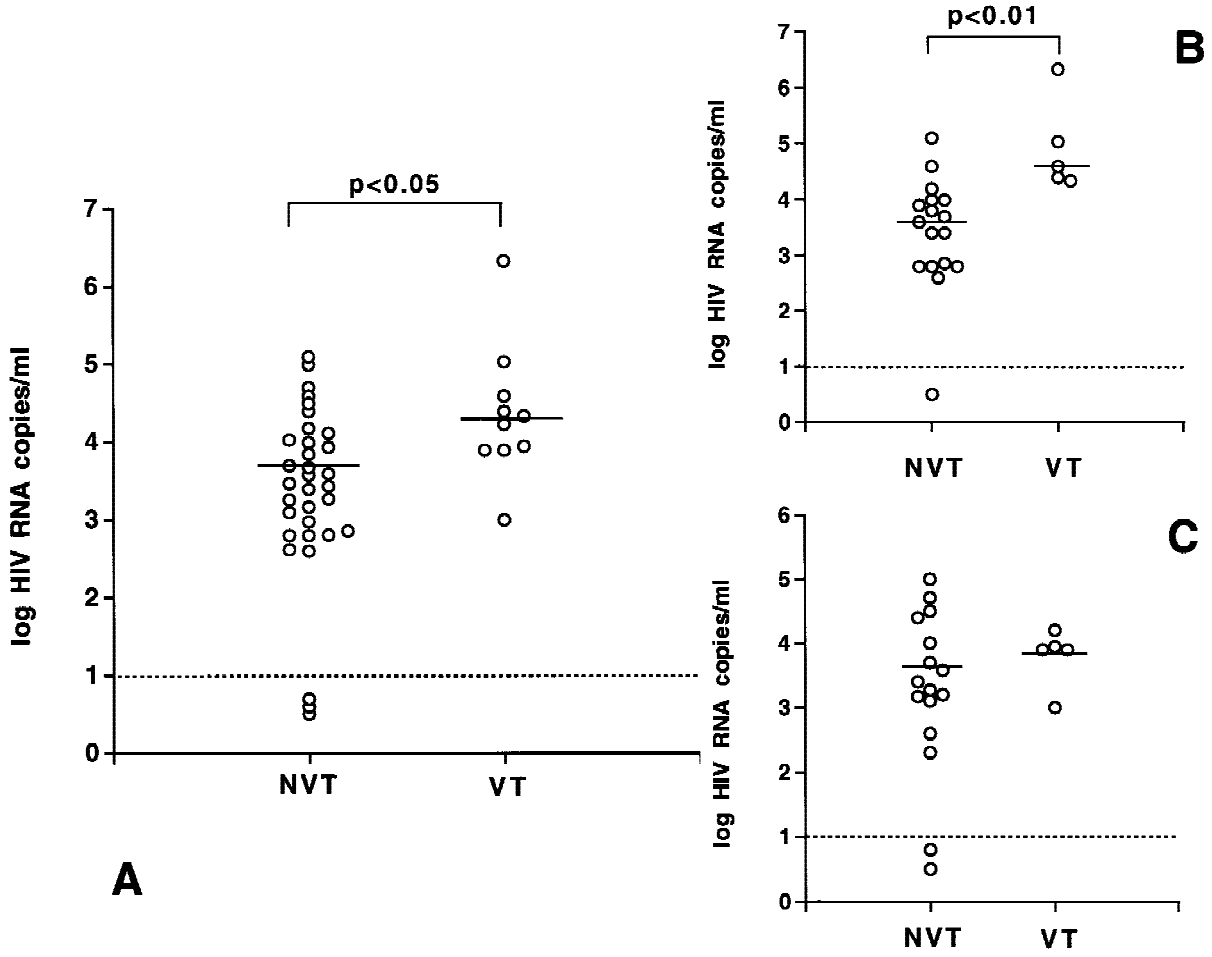

Fig. 1. **A:** HIV-1 RNA levels in mothers involved (VT) or not involved (NVT) in mother-to-infant HIV-1 transmission. **B:** Mothers with stage A1 (asymptomatic, CD4 counts ≥ 500 per mm^3). **C:** Mothers with stage A2 (asymptomatic, CD4 counts between 200 and 499 per mm^3) or A3 (asymptomatic, CD4 counts < 200 per mm^3) [Centers for Disease Control, 1992]. The dashed line represents the limit of sensitivity of the assay. Solid lines indicate median values.

tribution of HIV-1 RNA levels covered a wide range of concentration in both groups of mothers (Fig. 1A), but the 3 women resulting negative for the viral RNA were in the nontransmitting group. A threshold of 3.9 log copies/ml (about 8,500 copies/ml) enabled us to discriminate two groups with 10-fold different prevalence of HIV transmission. Nine of 20 women (45%) with HIV-1 RNA values ≥ 3.9 log copies/ml transmitted HIV-1 infection to their infants, whereas only 1 of 24 women (4.2%) with HIV-1 RNA values < 3.9 log copies/ml was involved in vertical transmission.

No difference in viral load was observed according to disease stage (A1 versus A2–A3) in the patient population. Among transmitting mothers, CD4 cell counts at delivery were directly correlated with HIV-1 RNA level ($P = .008$). The relationship between RNA levels and risk of transmission was strong in mothers with stage A1 ($P = .006$), whereas no difference in HIV-1 RNA load was observed between transmitting and nontransmitting mothers with stages A2–A3 ($P = .893$) (Figs. 1B and 1C). Among women with stage A1, a threshold level of 4.3 log copies/ml (20,000 copies/ml) had a positive predictive value of 71.4% and a negative predictive value of 100% over HIV-1 transmission.

DISCUSSION

Mother-to-child transmission of HIV-1 is the major route of infection in children with acquired immunodeficiency syndrome (AIDS). The prevalence of vertical-perinatal HIV-1 infection averages 14–40%, but the factors involved in the risk of transmission are not completely understood [Weiser et al., 1994; Dickover et al., 1996; European Collaborative Study, 1992, 1996; Landesman et al., 1996; Sperling et al., 1996; Burns et al., 1997; Cao et al., 1997]. A better knowledge of the correlates of mother-to-infant transmission of HIV-1 would provide relevant information for the development of preventive strategies.

The quantitative assessment of HIV-1 RNA represents a sensitive and specific method for the determination of HIV-1 load [Fang et al., 1995]. Recent reports indicate a statistical correlation between maternal HIV-1 RNA levels and risk of mother-to-child transmission [Fang et al., 1995; Dickover et al., 1996; Sper-

ling et al., 1996; Burns et al., 1997; Cao et al., 1997]. However, transmission appears to occur across a wide range of RNA levels and from some mothers with undetectable plasma viremia [Sperling et al., 1996; Burns et al., 1997; Cao et al., 1997]. The identification of factors interfering with the predictive value of viral load over the risk of perinatal HIV-1 infection would be important for the clinical use of HIV-1 RNA determination.

Previous studies showed that low maternal CD4 cell levels could increase the risk of transmission [European Collaborative Study, 1992, 1996]. In the present study, we failed to detect a relationship between HIV-1 transmission and CD4 counts. The relatively high CD4 cell levels of the mothers included in the study might at least partly account for this result. In the attempt to define factors that may interfere with the relationship between viral RNA levels and risk of transmission, we found a direct correlation between CD4 cell counts at delivery and HIV-1 RNA level in mothers who transmitted the infection. A high viral load was associated strongly with transmission in mothers with A1 stage, whereas no correlation was observed in mothers with A2/A3 stage. This finding suggests that the predictive value of maternal HIV-1 RNA levels upon perinatal infection is high when CD4 levels are normal or slightly reduced, but becomes limited when immune suppression is more severe. This observation is consistent with recent results showing attenuated association between HIV-1 RNA level and transmission in women with advanced HIV-1 infection [Burns et al., 1997].

Our results indicate that the quantification of HIV-1 RNA is a sensitive tool to assess the risk of transmission from mothers with stage A1 infection. The determination of RNA load enabled identification of a subset of women with high risk of vertical-perinatal transmission despite early stage of HIV-1 infection.

ACKNOWLEDGMENTS

The authors are grateful to Roche Diagnostic Systems (Milano, Italy) for providing Amplicor HIV Monitor kits.

REFERENCES

Burns DN, Landesman S, Wright DJ, Waters D, Mitchell RM, Rubinstein A, Willoughby A, Goedert JJ. 1997. Influence of other maternal variables on the relationship between maternal virus load and mother-to-infant transmission of human immunodeficiency virus type 1. J Infect Dis 175:1206–1210.

Cao Y, Krogstad P, Korber BT, Koup RA, Muldoon M, Macken C, Song J-L, Jin Z, Zhao J-Q, Clapp S, Chen ISY, Ho DD, Ammann AJ, and the Arie Project Investigators. 1997. Maternal HIV-1 viral load and vertical transmission of infection: the Ariel project for the prevention of HIV transmission from mother to infant. Nature Med 3:549–552.

Centers for Disease Control. 1992. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR 41:1–19.

Dickover RE, Garratty EM, Herman SA, Sims MS, Plaeger S, Boyer PJ, Keller M, Deveikis A, Stiehm ER, Bryson YJ. 1996. Identification of levels of maternal HIV-1 RNA associated with risk of perinatal transmission: effect of maternal zidovudine treatment on viral load. JAMA 275:599–605.

European Collaborative Study. 1992. Risk factors for mother-to-child transmission of HIV-1. Lancet 339:1007–1012.

European Collaborative Study. 1996. Vertical transmission of HIV-1: maternal immune status and obstetric factors. AIDS 10:1675–1681.

Fang G, Burger H, Grimson R, Tropper P, Nachman S, Mayers D, Weislow O, Moore R, Reyet C, Hutcheon N, Baker D, Weiser B. 1995. Maternal plasma human immunodeficiency virus type 1 RNA level: a determinant and projected threshold for mother-to-child transmission. Proc Natl Acad Sci USA 92:12100–12104.

Landesman S, Kalish LA, Burns DN, Minkoff H, Fox HE, Zorrilla C, Garcia P, Fowler MG, Mofenson L, Tuomela R. 1996. Obstetrical factors and the transmission of human immunodeficiency virus type 1 from mother to child. N Engl J Med 334:1617–1623.

Rodriguez EM, Mofenson LM, Chang BH, Rich KC, Fowler MG, Smeriglio V, Landesman S, Fox HE, Diaz C, Green K, Hanson IC. 1996. Association of maternal drug use during pregnancy with maternal HIV culture positivity and perinatal HIV transmission. AIDS 10:273–282.

Sperling RS, Shapiro DE, Coombs RW, Todd JA, Herman SA, McSherry GD, O'Sullivan MJ, Van Dyke RB, Jimenez E, Rouzioux C, Flynn PM, Sullivan JL, for the Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. 1996. Maternal viral load, zidovudine treatment, and the risk of transmission of human immunodeficiency virus type 1 from mother to infant. N Engl J Med 335:1621–1629.

Weiser B, Nachman S, Tropper P, Hawkins Viscosi K, Grimson R, Baxter G, Fang G, Reyelt C, Hutcheon N, Burger H. 1994. Quantitation of human immunodeficiency virus type 1 during pregnancy: relationship of viral titer to mother-to-child transmission and stability of viral load. Proc Natl Acad Sci USA 91:8037–8041.